Neurons and Behavior: Ex Uno, Plures

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http://dx.doi.org/10.1016/j.cell.2014.10.030

Li et al. demonstrate that a single interneuron can regulate analog- and digital-like behaviors guided by two different postsynaptic neurons. Releasing a single neurotransmitter onto downstream neurons that express receptors with distinct biophysical properties enables a small set of neurons to direct a range of functional responses.

Within the brain, individual neurons can accept input from many informational sources, and similarly, single neurons may then project to multiple postsynaptic targets. How these converging and diverging circuits regulate behavioral output is a major question in neuroscience research. It has long been appreciated that even relatively simple behaviors can involve distributed activity across many neurons, and individual neurons can regulate multiple behaviors (Briggman and Kristan, 2008). As the only animal with a complete neuronal roadmap, a connectome (White et al., 1986), the roundworm *C. elegans* provides a premier platform to examine how well-defined neuronal circuits regulate different and complex behaviors (Gray et al., 2005). Many of its 302 neurons are multifunctional, raising the question of how they operate at the circuit level. In this issue of *Cell*, Li and colleagues (2014) shed light on the mechanism through which a multifunctional neuron regulates multiple behaviors, applying an impressive array of experimental approaches, including in vivo calcium imaging in freely-behaving animals, imaging combined with simultaneous optogenetic circuit manipulation, and patch clamp electrophysiology.

As a first pass at understanding how multiple behaviors are regulated by relatively few neurons in *C. elegans*, the authors focused on locomotion. Among the neurons regulating locomotor behavior, the AIY interneuron was found to regulate both reversal and speed of locomotion. Interestingly, the activity of AIY, measured by calcium imaging in freely-behaving animals, exhibits a strong correlation with both forward speed (positive) and reversal initiation (negative). Furthermore, optogenetic activation or inhibition of AIY modulates speed and reversal initiation bidirectionally. To determine how AIY could regulate both behaviors at the circuit level, the authors examined three neurons directly downstream: AIZ, RIA, and RIB. They found that AIY controls reversal initiation by *inhibiting* AIZ and modulates speed by *activating* RIB (Figure 1). Thus, AIY regulates both forward speed and reversal via opposing effects on two downstream neurons.

To probe how AIY bidirectionally modulates RIB and AIZ, Li and colleagues examined the synaptic communication between AIY and the downstream neuron pair. They confirmed that AIY releases acetylcholine (ACh), which acts on both downstream neurons. Since AIY exerts the opposite effect on RIB and AIZ, apparently via the same neurotransmitter, each neuron would be expected to express different types of ACh-gated channels with divergent biophysical properties. Indeed, further experiments revealed that RIB expresses ACR-16/UNC-29, while AIY expresses ACC-2. In RIB, both ACR-16- and UNC-29-containing receptors were necessary for the modulation of locomotion speed by AIY. Likewise, in AIZ, reduction or elimination of ACC-2 eliminated the ability of AIY to generate reversals. ACC-2 is a type of ACh-gated Cl^- channel that has been described in *C. elegans* (Putrenko et al., 2005). When the channel is activated by ACh, only Cl^- ions flow into the cell, hyperpolarizing it. Thus, expression of this channel in AIZ renders the cholinergic AIY-AIZ synapse inhibitory.

The control of both speed and reversal by the same neuron is particularly intriguing because these behaviors are modulated in a fundamentally different way: speed is an analog behavior with a wide dynamic range, while initiation of reversal is digital. The authors probed how AIY drives these outputs with a series of electrophysiology, calcium imaging, and optogenetic experiments. Importantly, they established a causal link between AIY activity and the dynamic range of AIY and RIB responses by imaging calcium activity in AIZ and RIB while manipulating the activity in AIY optogenetically across a range of stimulus intensities. As AIY was more strongly inhibited, calcium activity in RIB gradually decreased in parallel over a wide dynamic range. In contrast, increasing optogenetic inhibition of AIY produced no effect on calcium in AIZ until a certain a threshold was reached, when the calcium in AIZ abruptly increased. Thus, RIB responded linearly across a wide dynamic range, while AIZ responses were more binary. This critical difference between AIZ and RIB correlates well with the ligand sensitivity of the ACh-gated channel(s) present in each neuron. It also provides the appropriate output signals that allow graded activity in AIY to drive both an analog-like behavior (speed, via RIB) and digital-like behavior (reversal, via AIZ).

A major implication of this study is that nonlinear responsiveness across different neurons can allow relatively few

upstream neurons to drive multiple, distinct behavioral responses. While these findings offer significant insight into how small nervous systems generate complex behaviors, they may be broadly applicable, as many neurons in animals with more complex nervous systems are multifunctional as well. Divergent output, coupled with differential responses across output pathways, could represent a network motif that is repeated across brain regions and taxa (Milo et al., 2002). Indeed, it appears to be analogous to the way dopaminergic circuits in *Drosophila* modulate the mushroom body. In this case, dopaminergic neurons innervate multiple different sets of downstream mushroom body neurons. Some of these neurons are differentially "tuned," exhibiting unique cellular responses to broad dopamine release (Boto et al., 2014). Similarly, in rodents, a large proportion of neurons in the amygdala receive information about sensory stimuli that drive fear conditioning, but relatively few neurons end up incorporated into the memory trace (Yiu et al., 2014). This again suggests differential responsiveness among the set of postsynaptic neurons. Nonlinear responses across multiple postsynaptic neurons to relatively uniform input stimuli could serve to drive distinct behavioral responses. Such network architecture has the potential to facilitate complex behaviors while minimizing the amount energetically-expensive neuronal circuitry required.

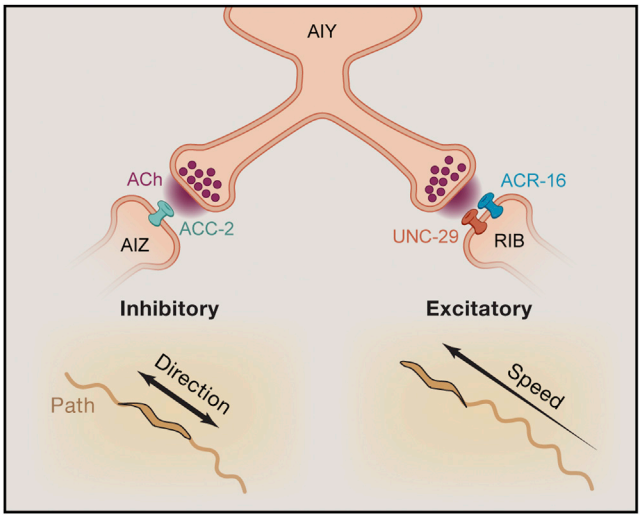

Figure 1. The AIY Neuron in *C. elegans* Regulates Two Locomotor Behaviors

Reversal is regulated through a narrow dynamic range, inhibitory cholinergic synapse with the downstream neuron AIZ. In addition, AIY regulates forward speed via a wide dynamic range, excitatory cholinergic synapse onto the RIB neuron. This architecture allows the single AIY neuron to regulate both analog- and digital-like behaviors by releasing the same neurotransmitter onto two different output neurons, each of which expresses acetylcholine (ACh) receptors with different biophysical properties.

On a broad level, the present study by Li et al. represents a stunning example of the power of *C. elegans* to answer fundamental questions about neuronal circuit function, parsing precise roles for individually identified neurons and their synaptic partners. It also highlights the importance of functional studies in predicting the behavioral roles of even small circuits. Considering just ACh neurotransmission, the *C. elegans* genome encodes 32 nicotinic AChRs subunits (Holden-Dye et al., 2013), four ACh-gated Cl^- channel subunits (Putrenko et al., 2005), and three muscarinic receptors (Lee et al., 2000). Therefore, ACh release will exert different effects on postsynaptic neurons depending on the ACh receptors that are present. In recent years, many groups have begun investing significant resources in connectomics, aiming to map all of the neuronal connections in a brain (or brain region). These investments are worthwhile and will no doubt yield significant insight. Nonetheless, the study by Li et al. highlights that, like the human genome, the human connectome will be a necessary, but not sufficient, condition for a complete understanding of brain function.

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